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Role of humic substances in agriculture and variability of their content in freshwater lake sapropel

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Abstract. The term 'humic substances' (HS) refers to a general category of naturally occurring, biogenic, heterogeneous organic substances. They create the most widespread natural organic matter found in sediments, soils and waters. Organic carbon in soil (up to 70%) and peat (up to 90%) usually occurs in the form of HS. HS influence the formation process of fossil fuels, as well as they are involved in the plant nutrition process, have an influence on availability and toxicity of metallic and other elements. Furthermore, HS play a significant role in the global carbon geochemical cycle. Properties and application efficiency of humus depend on the source of HS. Freshwater sapropel is a huge reservoir of HS with superior biological activity, although their total content is lower than in peat. The aim of this paper, firstly, was to present the information about the options of HS in agriculture and their main effects on plant growth. Secondly, determination and characterization of HS content in freshwater lake sapropel was performed as sapropel nowadays becomes a popular natural organic-mineral fertilizer and soil conditioner. Sapropel samples were derived from Lake Pilvelis, Lake Pilcines, Lake Vevers, Lake Liducis and Lake Padelis situated in Eastern Latvia. Investigation of HS content in sapropel is significant for the Baltic States and Northern Europe due to wide distribution and availability of sapropel in freshwater bodies. That promotes a search for new ways of extraction methods and bioeconomically effective utilization of this natural resource, obtainable in economically significant amounts, with high opportunities of its use especially in agriculture. Contemporary agriculture strongly desiderates in new products of high effectivity enhancing soil and crop productivity and quality hand in hand with sustainable development and careful attitude to the nature and surrounding environment, thus, one of the ways how it can be achieved is understanding how, where and how much HS preparations can be applied.

Key words: humic substances, freshwater sapropel, organic fertilizer, bio-stimulant, soil conditioner.

INTRODUCTION

The most widespread natural organic substances are humic substances (HS) containing up to 90% of dissolved organic carbon in the aquatic environment and up to 70% of organic carbon in the terrestrial environment (Kļaviņš, 1997). HS form large pools of the organic component of soils (Kelleher & Simpson, 2006), peat (Šīre, 2010; Purmalis, 2015), composts (Lima et al., 2010) and sapropel (Rūtiņa et al., 2013).

HS are formed during the humification process, i.e., decay of living matter (organic carbon reservoir) in an intermediate phase of the transformation process of organic carbon in the organic carbon cycle, or are deposited in fossil materials (Kelleher & Simpson, 2006; Šīre, 2010). Humification begins immediately after the downfall of an organism when easily degradable substances in cytoplasm are destroyed by extracellular ferments and ferments bound in cell membrane (MacCarthy, 2001). After the decomposition of the cell membrane, microorganisms start to participate in the process of humification; they are involved in breakdown and utilisation of carbohydrates, lipids, nucleic acids, proteins which are the components of the labile fraction of organic matter (Prentice & Webb, 2010; Ziechmann et al., 2000).

Formation of HS is influenced by many factors from which as the most important following can be mentioned: climate, composition of residues to be humified (parent material), dominant group of destructive organisms, pH, oxidation-reduction conditions, presence of oxygen, catalysts or inhibitors of humification (Eglīte, 2007; Kukuļs, 2018), for example, even newly formed HS itself can inhibit degradation and humification of the remains (Ziechmann et al., 2000).

Commonly HS are subdivided into three fractions depending on their solubility: humic acids (HA), fulvic acids (FA) and humins (Eglīte, 2007). Several terms summarized and explained in Table 1 will help to understand structural and functional properties of HS. It is assumed that fractions of humic substances contain organic compounds with similar general properties, while their structure, composition and specific properties in various environmental conditions may differ (Hayes, 1997).

Table 1. Important terms used in the studies of humic substances (MacCarthy et al., 1990; Heiri et al., 2001; Perminova et al., 2003; Vassilev et al., 2012)

Term	Definition
Organic matter	Total amount of the organic matter which is oxidised to carbon dioxide and ash at 500–550 °C during the first reaction of the loss-on-ignition method.
Humus (Soil organic matter)	Total amount of the heterogeneous organic substances in soil with low molecular weight (non-humic substances) and substances with high molecular weight (humic substances), except the soil biomass, undecomposed and partially decomposed products of plant and animal tissues.
Humic substances	The category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized by colour (yellow to black), and of high molecular weight and refractory.
Humic acids	Fraction of humic substances that is soluble in water at pH > 2 and insoluble in water under pH < 2. Molecular weight of different humic acids ranges approximately 15–20 kDa.
Fulvic acids	Fraction of humic substances soluble in water under all pH conditions. Molecular weight of fulvic acids of different origin ranges approximately 0.5–15 kDa.
Humins	Fraction of humic substances insoluble in water, alkali and acid. Humins are the most resistant to decomposition of all other fractions of humic substances. They play important role in water holding capacity, structure, stability and fertility of soil.
Humates	Mineral salts of soluble humic substances fractions.

General definition of humic substances determine them as high molecular weight compounds that together form brown to black coloured substances formed by secondary synthetic reactions and which do not belong to substance's classes of biochemistry such as amino acids, carbohydrates, fats, waxes, resins and organic acids. The term is used as a generic name to describe coloured material or its fractions obtained on the basis of solubility characteristics as described in Table 1 (Stevenson, 1994).

Fulvic acids usually form low-molecular weight fraction of HS with low concentration of aromatic groups and high concentration of carboxyl groups (Thomsen et al., 2002; Eglīte, 2007).

Humic acids are complex of molecules where amino acids, amino sugars, peptides and aliphatic compounds are incorporated in the aromatic structures (Purmalis, 2015; Kukuļs, 2018).

Humins are insoluble components of HS that remain after the extraction of all other substances soluble in aqueous base. The major components of humins are aliphatic hydrocarbon functionalities predominantly, but in small amounts carbohydrates, peptides and peptidoglycans. However, composition of humins differs considerably from the base-soluble components of humus (Hayes et al., 2017).

The aim of this paper, firstly, is to present an overview about the options of HS in agriculture and their main effects on plant growth. Secondly, determination and characterization of HS content in freshwater lake sapropel was performed as sapropel nowadays becomes a popular natural organic-mineral fertilizer and soil conditioner.

MATERIALS AND METHODS

Sediment sampling

To implement this study, 191 freshwater lake sediment samples from full profiles of five lakes (Padelis, Pilcine, Pilvelis, Liducis, Vevers) located in Eastern Latvia were collected (Fig. 1). Derived sediment samples were recognized predominantly as sapropel.

Figure 1. Freshwater sediment sampling sites in Eastern Latvia: (A) Lake Padelis, (B) Lake Pilcine, (C) Lake Pilvelis, (D) Lake Liducis, (E) Lake Vevers.

Sediment profiles were obtained using a 10 cm diameter Russian-type peat sampler with a 1.0 m long camera. Sediment monoliths were subsampled with interval 10 cm, homogenized and placed into plastic bags for transportation to the laboratory according to previously developed methodology (Givelet et al., 2004).

Sediment characterization

Assessment of sediment properties is more significant for characterization of HS in sapropel than distribution by sampling sites (BSSC Institute, 2010; Kurzo, 2005; Stankevica et al., 2015); therefore, all sapropel samples were classified applying sapropel type classification methodology (Stankeviča et al., 2017) as shown in Table 2. Furthermore, sapropel samples of organic and organic silicate types were subdivided considering the origin of organic matter in sapropel, i.e., algal and water animals (OS_{alg}, OSS_{alg}) and vascular, thelmatic plants (OS_{pla}, OSS_{pla}).

Table 2. Type and quantity of analysed sediment samples

Abbreviation	Characteristic type	Number of samples
OS	Organic sapropel	98
OSS	Organic silicate sapropel	61
CS	Carbonate sapropel	19
SS	Silicate sapropel	13

For classification of sediments the loss-on-ignition (LOI) method was applied in order to estimate content of ashes, organic matter and carbonate matter in the samples (Dean, 1974; Heiri et al., 2001), concentration of Ca and Fe was estimated using flame atomic absorption spectrometry equipment (Perkin Elmer 503). Reliability and accuracy of the analytical results were checked using blank and reference samples (ISE 1998.3-921 (Wageningen Evaluating Programmes for Analytical Laboratories), SLRSS-2 river water, BCSS- coastal marine sediments (Analytical Chemistry Standards NRC, Canada)).

Origin and composition of sediment organic matter were identified using Motic DM-B1 Digital Microscope with magnification by 400–1,000 times.

Extraction of humic substances, isolation of humic and fulvic acids, detection of total organic carbon

HS from sediment samples were extracted using conventional extraction technique such as low temperature treatment procedure; humic and fulvic acids were isolated and purified using techniques recommended by the International Humic Substances Society (Tan, 2005). Briefly, 0.5 g of air-dried and finely grounded sapropel sample was treated in N_2 with 25 mL of 2% NaOH for 24 h applying stirring. Obtained suspension was filtered, and 1 mL of it was diluted by 100 times; subsequently, absorption at 410 nm was measured to estimate total content of HS. Afterwards, the remaining solution was acidified with concentrated HCl to pH < 2 to precipitate humic acids, leaving fulvic acids in the solution (Tan, 2005).

Calculation of the content of humic acids and fulvic acids was performed using a calibration method. For each lake calibration equations for both, humic and fulvic acids, were done individually.

Total organic carbon of HS was detected using TOC-V CSN analyser (Shimadzu).

Data statistical analysis and data visualisation

Correlation analysis with a data set (N = 178) of humic substances, total content of humic, fulvic acids and quantity of total organic carbon in humic substances was performed using the MS Excel correlation analysis tool. Statistically significant correlation was applied with probability lower than 5% ($P < 0.05$) if critical value for the Pearson's correlation coefficient was $r\ 0.05 = 0.15$. Obtained data were plotted and visualised using the Adobe Illustrator CC.

RESULTS AND DISCUSSION

Overview of humic substances' applicability in agriculture

Contemporary ways of soil cultivation in agricultural practice lead to faster decomposition of humus, including humic substances, therefore, soil enrichment with humus in natural way occurs too slowly (Rode et al., 1993). HS in environment can be found even in depleted soils at various concentrations, but their impact on living organisms and plants is not as intensive as it appears from refined preparations made from purified HS and humates – in literature they are named as biostimulants (Canellas et al., 2015) and plant regulators (Baldotto & Baldotto, 2014). This difference occurs due to the fact that HS in soil exist in the form of inactive or low-activity compounds (Eshwar et al., 2017). Functional groups of HS may actively react with mineral components of soil such as cations, oxides, hydroxides, aluminosilicates; as a result, these functional groups are bound and blocked leading to decreased physiological activity and elevation of ashiness (Orlov, 1993; Dūdare, 2015).

During the production of preparations from humates and HS the state of HS is changed. By elimination and purification of HS from mineral components their functional groups are released that allows involvement of HS in various chemical and biochemical processes. Furthermore, during the process of production, molecules are partly destructed that increases amount of free radicals and distribution of molecular mass resulting in enhancement of low molecular fraction. Both, increase of free radicals and decrease of molecular mass of HS, facilitate entrance of humates and other kind of HS preparations into plant tissues and involvement in biochemical processes (Orlov, 1993).

HS form 85–90% of humus or soil organic matter, while the remaining 10–15% are amino acids, carbohydrates, fats, waxes, resins and organic acids (Orlov et al., 1996). Properties and composition of humic substances vary in large ranges depending on many factors including parent material. In biosphere HS play vital role in sustainable life processes as they are involved in common functions like element accumulation, transportation, regulation, protection and regulate physiology of living organisms (Table 3).

Furthermore, HS preparations have accelerating effect on the production cycle of plants as well as enlarge initial growth rates while plants acclimatize to new conditions acting especially effectively on root system (Hernandez et al., 2015). In addition, HS act as carriers for beneficial microorganisms in cropping system (Canellas et al., 2015). All these findings are significant to rise popularity for HS to be used in various agricultural practices.

Table 3. Functional mechanism of humic substances (HS) in nature and their effects on plants (Orlov, 1993; Orlov et al., 1996; Trevisa et al., 2010; Canellas et al., 2015; Palanivell et al., 2015; Olaetxea et al., 2018; Yildiztekin et al., 2018)

HS function	HS mechanism	HS effect	HS fraction
1. Accumulation in soils, natural waters and sediments where HS are a source of energy and nutrition for biota	1. Long-term deposition (in the form of HS) of nutritional elements and organic compounds with high energy value.	1.1. Accumulation of 90–99% of soil total N, more than 50% of total P, S. 1.2. Long-term remain of K, Ca, Mg, Fe, almost all necessary microelements for microorganisms 1.3. Determination of cation exchange capacity 1.4. Plant nutrition regulation	Humic acids Humins
2. Protection of soil biota, vegetation and groundwater	2. Incorporation of radionuclides, detergents, pesticides	2.1. Soil resistance elevation of dryness, wetting, erosion and deflation 2.2. Soil resistance of technogen loads 2.3. Decrease plant toxicity of heavy metals pollution 2.4. Groundwater protection from mineral and organic pollutants	Humic acids Humins
3. Transformation in soil, sediments and waters	3. Cation metallic elements (except alkali and some alkaline earth metals) migration form as complex organic-mineral compounds with fulvic acids ligands	3.1. Metallic element migration in environment at any pH 3.2. Unavailable soil mineral resolution for living organisms 3.4. Determination of cation exchange capacity 3.3. Plant nutrition regulation	Fulvic acids
4. Regulation of almost all necessary properties of soil	4.1. Setting down of soil colour	4.1. Regulation of soil temperature regime	Humic acids Humins
	4.2. Reflection of small amount of solar energy	4.2. Effective soil heating, elevating of soil heat capacity, decrease thermal conductivity	Humic acids
	4.3. Binding of soil particulate elements	4.3. Formation of soil structure	Humic acids Humins
5. Physiological regulation of live organisms and plants	5.1. Amino acids, vitamins and antibiotics transportation	5.1. Alleviate adverse conditions effects on plants and live organisms	Humic acids Fulvic acids
	5.2. Auxin-like activity	5.2. Increase plant root, shoot development and plant production of dry matter	Humic acids Fulvic acids

Maximum efficacy at minimum costs is the main principle on which farmers rely when evaluating utility and efficiency of any preparation applicable in agriculture for soil improvement. If assessing a possibility of direct applications of HS into soil, then according to the data derived at Alibaba.com price of HS varies from 80 EUR to

1750 EUR per ton. The price mainly is dependent on used raw material and producer country, i.e., HS derived from coals are cheaper than those from peat, and the cheapest HS preparations are produced in China. At HS application rate of 100 mg kg^{-1}, approximately equivalent to 100 kg ha^{-1} in topsoil (incorporation in top 10 cm) (Rose et al., 2014), costs may vary from 8 EUR to 175 EUR per hectare, but using liquid preparation of HS for watering or spraying of plant the expenses are significantly lower.

As it was stated previously, HS themselves are not a fertilizing agent because do not contain nutrients necessary for plants in sufficient quantities and in the right proportions. However, they are involved in various functional processes, e.g., promote accumulation of mineral substances in form of humates in soil and stimulate uptake of these elements into plant tissues (Table 3). Such a wide range of direct and indirect impacts of HS in open uncontrolled field environment leads to ambiguous results. Some studies reveal that yield growth at open fields does not exceed 5% that actually is a range of uncertainty in agricultural studies. However, in general, the studies reveal that biostimulants derived from HS cause positive influence on development of horticultural and ornamental plants, i.e., shoot and root growth can be increased by 15–25% for various plant species depending on the application form of preparation (Rose et al., 2014). Moreover, HS act more effectively on monocotyledonous than on dicotyledonous plants after spraying plants, not applying into growing media. The reason for such impact is not investigated in details (Canellas et al., 2015). Significant is also a nature of HS. Plant physiological response is stronger to HS which are derived from non-charred sediments such as peat, compost, biohumus and sapropel in comparison to brown coal, leonardite, subbituminous coals (Canellas & Olivares, 2014). Another study revealed that HS which are derived by composting solid household waste in a mixture with waste water from olive oil production could be used as valuable biostimulants in agricultural practices due to their capacity to promote plant growth, activity of marker enzyme (nitrate reductase, glutamine synthase, phosphoglucose isomerase and pyruvate kinase), the amount of total proteins and nutrient accumulation (Palumbo et al., 2018). Such HS applied on maize at concentration 0.5 mg C L increased the dry weight of leaves and roots by 32–68%; but higher concentrations did not affect plant growth significantly. Furthermore, at tested concentration of HS elevated content of micro- and macroelements was detected in leaves and roots (Palumbo et al., 2018).

Experiment with HS preparation derived from vermicompost revealed that its spray applications on lettuce at concentration 15 mg C L resulted in shorter harvesting period (reduced by 6 days), increased number of leaves (on average by 4 leaves), increased content of enzymes that are involved in sugar metabolism and reduced content of carbohydrates. Lettuce after HS applications was growing healthier and thicker (Hernandez et al., 2015).

Assessment of humic substances' content in sapropel

Sapropel, also called as 'gyttja' or 'dy' (Hansen, 1959), is a type of sediments formed from the remains of water plants, plankton and benthic organisms which undergo the transformation performed by microorganisms and by chemical and physical reactions (usually at oxygen-free conditions), as well is mixed with mineral components supplied from the water basin (Vincevica-Gaile & Stankevica, 2018). Sapropel is a renewable natural resource with a wide range of possible application ways in broad spectrum of fields of national economics, among which agriculture currently takes the greatest part

(Stankevica et al., 2016). Besides the investigation of sapropel's composition, HS content in sapropel has not been studied widely. N. Braks in his research mentioned HS as a component that increase a binding property of sapropel which was experimentally tested in production of sapropel concrete and a binder for production of wood chipboard (Braks et al., 1960; Braks, 1971). Another direction of sapropel application has been studied by Russian and Byelorussian scientists who investigated HS of sapropel with the aim to apply sapropel in balneology and for production of new medication and food or feed supplements (Kosyanova et al., 1993; Kurzo, 2005; Kitapova & Ziganshin, 2015) as sapropel contains smaller amount of HS in comparison to other sediments (soil, peat, biohumus etc.) but biological activity of sapropel's HS comparably is higher. HS of sapropel has high ability of biogenic stimulation, i.e., they stimulate macrophagial protection reactions, promote tissue repair processes, cause anti-inflammatory effect in cases of burns and corneal disease. They also stimulate breathing and inhibits activity of free radicals (Kurzo, 2005; Makarov et al., 2017). Therefore, nowadays the interest increases to produce preparations from sapropel that can be applied as biostimulants in various spheres, including human and veterinary healthcare, as well as agricultural use.

Previously performed studies (Bunere & Stankeviča, 2013; Bunere et al., 2014) revealed that humic acids derived from organic sapropel applied together with mineral fertilizers positively influence plant growth at hydroponic conditions. The optimum concentration of purified humic acids that were applied on plants was 5 mg L^{-1}; such applications resulted in longer leaves, greater dry mass of roots and leaves as well as higher concentration of chlorophyll in leaves. However, the concentration that exceeded the optimum was acting oppositely, i.e., plant development was inhibited. It can be supposed that optimum concentrations of purified HS and humates that can positively affect plants and soil organisms in soil will be higher than for applications in hydroponics, and may vary depending on soil type because in soil they actively react with mineral components resulting in lower activity (Orlov, 1993).

Results of freshwater lake sapropel analyses revealed that total content of humic substances varied from 9 mg g^{-1} up to 106 mg g^{-1} in sapropel's organic matter, 2–67 mg g^{-1} in sapropel's dry matter, and 0.3–6.0 mg g^{-1} of bulk sapropel mass (Table 4).

Obtained results indicated that the highest total content of HS (80–106 mg g^{-1}) from organic matter and its fraction of humic acids can be found in sediments which genetically are similar to peat (OS_{pla}, OSS_{pla}) and which organic matter mainly consists of higher plants (vascular plants) (Fig. 2A). Such HS contain high content of humic acids (70–95 mg g^{-1}), and HA/FA ratio is greater than 6 (Table 4).

Table 4. Average content and characteristics of humic substances in studied sapropel samples, mg g^{-1} (BS – bulk sediments, DM – dry matter, OM – organic matter, TOC – total organic carbon, HA/FA – humic acids/fulvic acids)

Sediment type	Humic substances			Humic acids			Fulvic acids			HA/FA	TOC
	BS	DM	OM	BS	DM	OM	BS	DM	OM		
OS_{alg}	1	26	34	1	21	27	0.4	6	7	4	2,417
OS_{pla}	5	69	81	4	61	72	0.6	8	10	9	622
OSS_{alg}	2	25	42	2	20	34	0.4	5	8	4	5 461
OSS_{pla}	6	63	106	5	56	95	0.6	7	11	10	871
CS_{alg}	0.3	2	9	0.2	1	5	0.2	1	4	1	1,820
SS_{alg}	2	12	43	2	10	36	0.4	2	7	5	9,512

On the contrary, in sapropel's HS which organic matter consists only of algae and animal remains humic acids can be detectable at low levels, and HA/FA ratio is below 5 (Kurzo, 2005; Shtin, 2005).

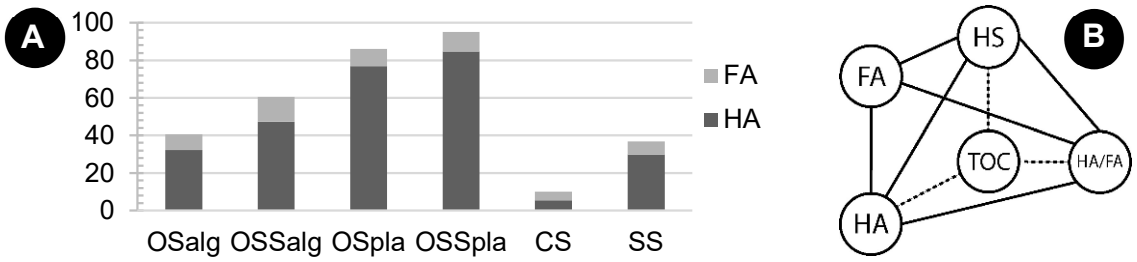

Figure 2. a) Content of humic substances and their fractions in different types of sapropel; b) Statistically significant correlation ($P < 0.05$, $N = 178$) of humic substances (HS), total content of humic acids (HA), fulvic acids (FA) and quantity of total organic carbon (TOC) in humic substances: (line) positive correlation, (dots) negative correlation.

In general, content of humic acids in sapropel's organic matter from algae decreased by 70 times (in comparison to sapropel's organic matter from vascular plants), while content of fulvic acids decreased by 3 times, and varied in all tested sediment samples in comparatively constant range from 4 mg g^{-1} to 12 mg g^{-1}, but total organic carbon concentration was assessed as high (1820–9512 mg g^{-1} HS).

Significant correlation ($P < 0.05$, $N = 178$) was determined regarding total organic carbon, i.e., for samples of sediments with higher total content of humic substances and humic acids, as well as HA/FA ratio and total organic carbon content was lower (Fig. 2, B). Poor correlation was detected for humic substances or their fractions and organic matter content.

CONCLUSIONS

Investigation of HS content in sapropel is significant for the Baltic States and Northern Europe due to wide distribution and availability of sapropel in freshwater bodies. That promotes a search for new ways of extraction methods and bioeconomically effective utilization of this natural resource, obtainable in economically significant amounts, with high opportunities of its use especially in agriculture.

Content of HS in organic rich lake sediments such as freshwater sapropel varies from 9 mg g^{-1} to 106 mg g^{-1}, humic acids: 5–95 mg g^{-1} and fulvic acids 4–11 mg g^{-1}. Sediments more rich in HS are those which consist from peat forming plants, but total organic carbon concentration is higher in HS which are formed from algae and animal remains.

Scientific literature reveals the evidence that HS of sapropel has higher biological activity than other HS (e.g., derived from peat), thus, sapropel's HS can become as a valuable raw material for balneology, human and veterinary healthcare, but in case of agriculture their extraction can be too expensive and uneconomical. For agricultural purposes application of HS derived from biohumus and compost would be more reasonable and efficient. Contemporary agriculture strongly desiderates in new products of high effectivity enhancing soil and crop productivity and quality hand in hand with

sustainable development and careful attitude to the nature and surrounding environment, thus, one of the ways how it can be achieved is understanding how, where and how much HS preparations can be applied, but these studies need to be continued.

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REFERENCES

Baldotto, L.E.B. & Baldotto, M.A. 2014. Adventitious rooting on the Brazilian red-cloak and sanchezia after application of indole-butyric and humic acids. *Horticultura Brasileira* **32**, 434–439.

Braks, N., Alksne, A., Āboliņš, J. & Kalniņš, A. 1960. Sapropel and peat humic acids as binder for wood residue use. *Latvijas PSR Zinātņu akadēmijas Vēstis* **10**, 101–108 (in Latvian).

Braks, N.A. 1971. *Sapropel sediments and application possibilities,* Riga, Zinatne, 282 pp. (in Russian).

BSSC Institute. 2010. Sapropel: industrial-genetic classification. *Environmental protection and nature use.* Minsk: Nedra, 9 pp. (in Russian).

Bunere, S. & Stankeviča, K. 2013. Effects of humic substances derived from sapropel on the groth of radish (*Raphanus sativus L.*). *International Workshop "Peat and Humic Substances and Their Application"*, University of Latvia, Riga, Latvia.

Bunere, S., Stankeviča, K. & Klavins, M. 2014. Effects of sapropel on the growth of radish (*Raphanus sativus L.*). *Proceedings of the 56th International Scientific Conference of Daugavpils University*, 16–24.

Canellas, L.P. & Olivares, F.L. 2014. Physiological responses to humic substances as plant growth promoter. *Chemical and Biological Technologies in Agriculture* **1**, 1–11.

Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D.L., Nebbioso, A., Mazzei, P. & Piccolo, A. 2015. Humic and fulvic acids as biostimulants in horticulture. *Scientia Horticulturae* **196**, 15–27.

Dean, W.E.J. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: Comparison with other methods. *Journal of Sedimentary Research* **44**, 242–248.

Dūdare, D. 2015. *Interaction of peat humic acids with metallic elements.* Doctoral Thesis, Riga, University of Latvia, 164 pp. (in Latvian).

Eglīte, L. 2007. *Humic substances, their interaction with soil components and immobilisatio of humic substances.* Doctoral Thesis, Riga, University of Latvia, 159 pp. (in Latvian).

Eshwar, M., Srilatha, M., Bhanu Rekha, K. & Harish Kumar Sharma, S. 2017. Characterization of humic substances by functional groups and spectroscopic methods. *International Journal of Current Microbiology and Applied Sciences* **6**, 1768–1774.

Givelet, N., Le Roux, G., Cheburkin, A., Chen, B., Frank, J., Goodsite, M.E., Kempter, H., Krachler, M., Nørnberg, T., Rausch, N., Rheinberger, S., Roos-Barraclough, F., Sapkota, A., Scholz, C. & Shotyk, W. 2004. Suggested protocol for collecting, handling and preparing peat cores and peat samples for physical, chemical, mineralogical and isotopic analyses. *Journal of Environmental Monitoring* **6**, 481–492.

Hayes, M.H.B. 1997. Emerging concepts of the compositions and structures of humic substances. In Hayes, M.H.B. & Wilson, W.S. (eds.): *Humic Substances, Peats and Sludges.* Woodhead Publishing, pp. 3–30.

Hayes, M.H.B., Mylotte, R. & Swift, R.S. 2017. Humin: Its composition and importance in soil organic matter. In Sparks, D. L. (ed.): *Advances in Agronomy.* Academic Press, pp. 47–138.

Hansen, K. 1959. The terms gyttja and dy. *Hydrobiologia* **13**, 309–315.

Heiri, O., Lotter, A.F. & Lemcke, G. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology* **25**, 101–110.

Hernandez, O.L., Calderín, A., Huelva, R., Martínez-Balmori, D., Guridi, F., Aguiar, N.O., Olivares, F.L. & Canellas, L.P. 2015. Humic substances from vermicompost enhance urban lettuce production. *Agronomy for Sustainable Development* **35**, 225–232.

Yildiztekin, M., Tuna, A.L. & Kaya, C. 2018. Physiological effects of the brown seaweed (*Ascophyllum nodosum*) and humic substances on plant growth, enzyme activities of certain pepper plants grown under salt stress. *Acta Biologica Hungarica* **69**, 325–335.

Kelleher, B.P. & Simpson, A.J. 2006. Humic Substances in Soils: Are They Really Chemically Distinct? *Environmental Science & Technology* **40**, 4605–4611.

Kitapova, R.R. & Ziganshin, A.U. 2015. Biological activity of humic substances from peat and sapropel. *Kazan Medical Journal* **1**, 84–89 (in Russian).

Kļaviņš, M. 1997. *Aquatic humic substances: Characterisation, structure and genesis,* Riga, 234 pp.

Kosyanova, Z.F., Orlov, D.S. & Ammosova, Y.M. 1993. Humic acids of peloids. In Orlov, D.S. & Frolova, A.A. (eds.) *Humic substances in the biosphere.* Moskow: Nauka, pp. 74–79 (in Russian).

Kukuļs, I. 2018. *Characterization of forest soil humus and humus development during afforestation of former agricultural lands in Latvia.* Doctoral Thesis, Riga, University of Latvia, 160 pp. (in Latvian).

Kurzo, B.V. 2005. *Regularities of sapropel formation and application*. Minsk, 224 pp. (in Russian).

Lima, C.C., Mendonça, E.D.S. & Roig, A. 2010. Contribution of humic substances from different composts to the synthesis of humin in a tropical soil. *Revista Brasileira de Ciência do Solo* **34**, 1041–1048 (in Spanish).

MacCarthy, P. 2001. The principles of humic substances. *Soil Science* **166**, 738–751.

MacCarthy, P., Malcolm, R.L., Clapp, C.E. & Bloom, P.R. 1990. An introduction to soil humic substances. *Humic Substances in Soil and Crop Sciences: Selected Readings.* Madison, Soil Science Society of America, pp. 261–271.

Makarov, S.V., Nikolaev, I.A. & Maksimyuk, N.N. 2017. Sapropel as humic acid source for biogenic stimulants manufacture. *Molodoy uchenyy* **20**, 170–172 (in Russian).

Olaetxea, M., De Hita, D., Garcia, C.A., Fuentes, M., Baigorri, R., Mora, V., Garnica, M., Urrutia, O., Erro, J., Zamarreno, A.M., Berbara, R.L. & Garcia-Mina, J.M. 2018. Hypothetical framework integrating the main mechanisms involved in the promoting action of rhizospheric humic substances on plant root- and shoot-growth. *Applied Soil Ecology* **123**, 521–537.

Orlov, D., Biryukova, O. & Sukhanova, N. 1996. *Soil organic matter of Russia,* Moskow, Nauka, 258 pp. (in Russian).

Orlov, D.S. 1993. Properties and functions of humic substances. In Orlov, D.S. & Frolova, A.A. (eds.) *Humic substances in the biosphere.* Moskow: Nauka, pp. 16–27 (in Russian).

Palanivell, P., Ahmed, O.H., Ab Majid, N.M., Jalloh, M.B. & Susilawati, K. 2015. Improving lowland rice (*O. sativa L.* cv. MR219) plant growth variables, nutrients uptake, and nutrients recovery using crude humic substances. *The Scientific World Journal* **14**, 1–14.

Palumbo, G., Schiavon, M., Nardi, S., Ertani, A., Celano, G. & Colombo, C.M. 2018. Biostimulant potential of humic acids extracted from an amendment obtained via combination of olive mill wastewaters (OMW) and a pre-treated organic material derived from municipal solid waste (MSW). *Frontiers in Plant Science* **9**, 1–14.

Perminova, I.V., Frimmel, F.H., Kudryavtsev, A.V., Kulikova, N.A., Abbt-Braun, G., Hesse, S. & Petrosyan, V.S. 2003. Molecular weight characteristics of humic substances from

different environments as determined by size exclusion chromatography and their statistical evaluation. *Environmental Science & Technology* **37**, 2477–2485.

Prentice, A.J. & Webb, E.A. 2010. A comparison of extraction techniques on the stable carbon-isotope composition of soil humic substances. *Geoderma* **155**, 1–9.

Purmalis, O. 2015. *Peat humic substances: composition and influencing factors of their formation.* Doctoral Thesis, Riga, University of Latvia, 158 pp. (in Latvian).

Rode, V.V., Alyautdinova, R.H., Ekaterinina, L.N., Ryzhkov, O.G. & Motovilova, L.V. 1993. Plant growth stimulants from brown coal. In Orlov, D.S. & Frolova, A.A. (eds.) *Humic substances in the biosphere.* Moskow: Nauka, pp. 162–166 (in Russian).

Rose, M.T., Patti, A.F., Little, K.R., Brown, A.L., Jackson, W.R. & Cavagnaro, T.R. 2014. A meta-analysis and review of plant-growth response to humic substances: Practical implications for agriculture. In Sparks, D.L. (ed.) *Advances in Agronomy.* Academic Press, pp. 37–89.

Rūtiņa, L., Stankeviča, K. & Kļaviņš, M. 2013. Variabilities of humic substances content in lake sapropel, Latvia. The 71th Scientific Conference of University of Latvia, Riga, University of Latvia, 469–470 (in Latvian).

Shtin, S.M. 2005. *Lake sapropels and those complex cultivation,* Moscow, Moscow State Mining University, 373 pp. (in Russian).

Stankevica, K., Kalnina, L., Klavins, M., Cerina, A., Ustupe, L. & Kaup, E. 2015. Reconstruction of the Holocene palaeoenvironmental conditions accordingly to the multiproxy sedimentary records from Lake Pilvelis, Latvia. *Quaternary International* **386**, 102–115.

Stankevica, K., Vincevica-Gaile, Z. & Klavins, M. 2016. Freshwater sapropel (gyttja): Its description, properties and opportunities of use in contemporary agriculture. *Agronomy Research* **14**, 929–947.

Stankeviča, K., Kļaviņš, M. & Kalniņa, L. 2017. Sapropel definition and possible classification. In Kļaviņš, M. (ed.) *Peat and sapropel - synergy between production, science and the environment in the context of resource efficiency.* Riga: University of Latvia, pp. 165–168 (in Latvian).

Stevenson, F.J. 1994. *Humus chemistry: Genesis, composition, reactions. Second edition,* New York, John Wiley & Sons, 512 pp.

Šīre, J. 2010. *Composition and properties of rised bog peat humic acids.* Doctoral Thesis, Riga, University of Latvia, 105 pp.

Tan, K.H. 2005. *Soil Sampling, Preparation, and Analysis. Second Edition,* New York, US, CRC Press, 680 pp.

Thomsen, M., Lassen, P., Dobel, S., Hansen, P.E., Carlsen, L. & Mogensen, B.B. 2002. Characterisation of humic materials of different origin: A multivariate approach for quantifying the latent properties of dissolved organic matter. *Chemosphere* **49**, 1327–1337.

Trevisan, S., Francioso, O., Quaggiotti, S. & Nardi, S. 2010. Humic substances biological activity at the plant-soil interface. *Plant Signaling & Behavior* **5**, 635–643.

Vassilev, S.V., Baxter, D., Andersen, L.K., Vassileva, C.G. & Morgan, T.J. 2012. An overview of the organic and inorganic phase composition of biomass. *Fuel* **94**, 1–33.

Vincevica-Gaile, Z. & Stankevica, K. 2018. Impact of micro- and macroelement content on potential use of freshwater sediments (gyttja) derived from lakes of Latvia. *Environmental Geochemistry and Health* **40**, 1725–1738.

Ziechmann, W., Hübner, M., Jonassen, K.E.N., Batsberg, W., Nielsen, T., Hahner, S., Hansen, P.E. & Gudmundson, A.L. 2000. Humic substances and humification. In Ghabbour, E.A. & Davies, G. (eds.) *Humic Substances.* Woodhead Publishing, pp. 9–20.